

Supporting Information

Biochemical and structural analysis of aminoglycoside acetyltransferase Eis from *Anabaena variabilis*

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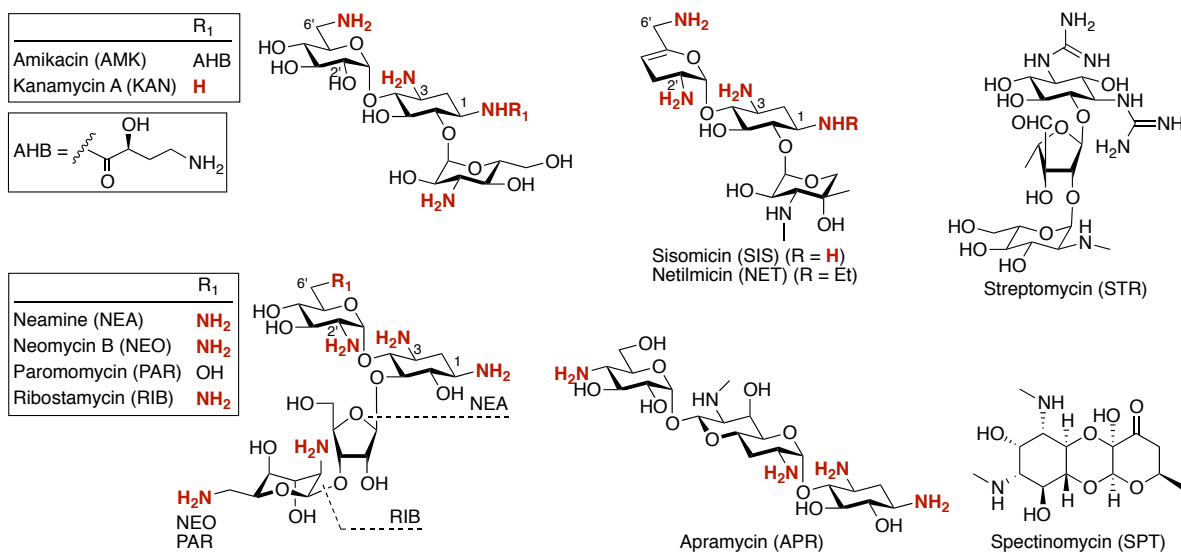


Fig. S1. Structures of aminoglycosides (AGs) tested in this study. The amine functionalities that could potentially be modified by Eis proteins are highlighted in bold red.

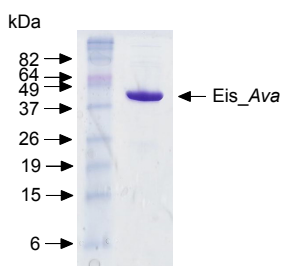


Fig. S2. 15% Coomassie blue-stained SDS-PAGE of purified Eis_Ava.

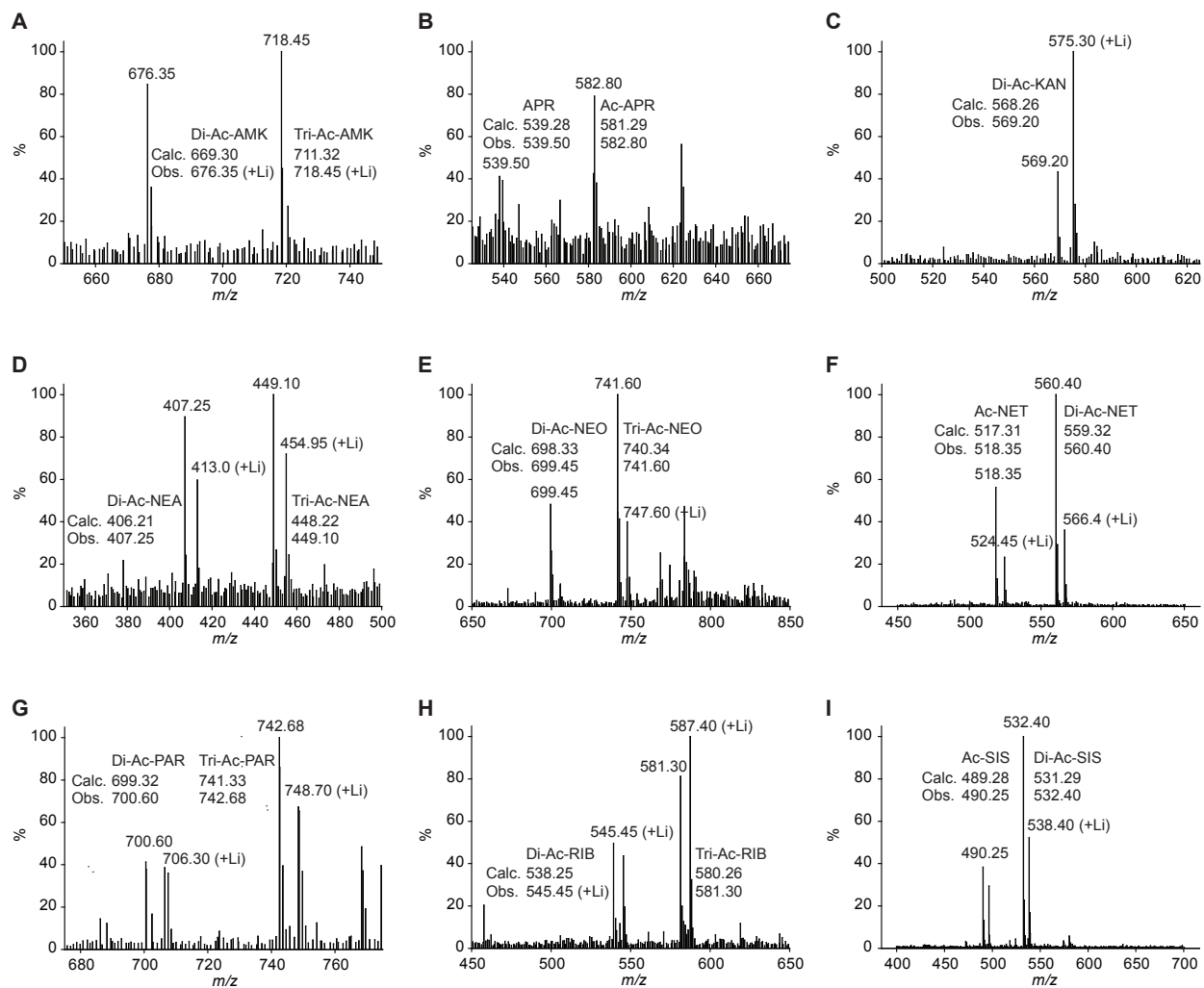


Fig. S3. Mass spectra of AGs multi-acetylated by *Eis_Ava*.

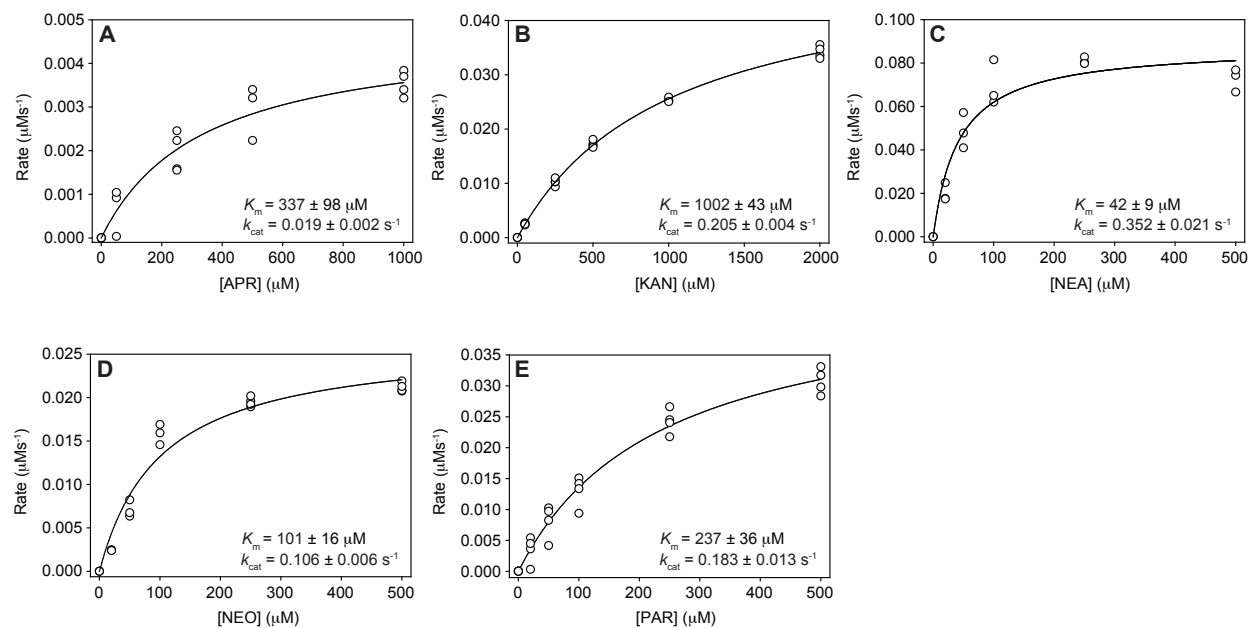


Fig. S4. Michaelis-Menten analysis of the Eis_Ava catalyzed acetylation of **A.** APR, **B.** KAN, **C.** NEA, **D.** NEO, and **E.** PAR.

Table S1. Proton chemical shifts determined for NEA and 1,2'-di-acetyl-NEA.^a

Ring	H position	NEA	1,2'-di-acetyl-NEA	Δppm
II	1	2.91-2.88 ^b (m) [2.89] ^d	3.70-3.84 (m) [3.77]	0.88
	2 _{ax}	1.33 (ddd (app. q), $J_{2ax,2eq} = J_{2ax,1} = J_{2ax,3} = 12.0$ Hz) ^c	1.72 (ddd (app. q), $J_{2ax,2eq} = J_{2ax,1} = J_{2ax,3} = 13.0$ Hz)	0.39
	2 _{eq}	2.08 (ddd (app. dt), $J_{2eq,2ax} = 12.0$ Hz, $J_{2eq,1} = J_{2eq,3} = 4.0$ Hz)	2.25 (ddd (app. dt), $J_{2eq,2ax} = 13.0$ Hz, $J_{2eq,1} = J_{2eq,3} = 4.1$ Hz)	0.17
	3	2.96 ^b (ddd, $J_{3,2eq} = 4.0$ Hz, $J_{3,2ax} = 12.0$ Hz, $J_{3,4} = 9.5$ Hz)	3.33-3.41 (m) [3.38]	0.42
	4	3.35 ^c (dd, (app. q) $J_{4,3'} = J_{4,5'} = 9.5$)	3.91-3.79 (m) [3.82]	0.45
	5	3.59-3.55 (m) [3.57]	3.66-3.58 (m) [3.62]	0.05
I	6	3.29-3.23 ^c (m) [3.26]	3.51-3.37 (m) [3.42]	0.16
	1'	5.42 (d, $J_{1',2'} = 4.0$ Hz)	5.76 (d, $J_{1',2'} = 3.8$ Hz)	0.34
	2'	2.87 (dd, $J_{2',1'} = 4.0$ Hz, $J_{2',3'} = 10.5$ Hz)	4.01-3.93 (m) [3.98]	1.11
	3'	3.63 (dd (app. t), $J_{3',4'} = 9.5$ Hz, $J_{3',2'} = 10.5$ Hz)	3.92-3.85 (m) [3.89]	0.26
	4'	3.35 (dd, (app. q) $J_{4',3'} = J_{4',5'} = 9.5$)	3.51-3.37 (m) [3.44]	0.09
	5'	3.91 (ddd, $J_{5',4'} = 9.5$ Hz, $J_{5',6'a} = 7.5$ Hz, $J_{5',6'b} = 3.0$ Hz)	4.01-3.93 (m) [3.96]	0.05
Acetyl	6' _a	3.04 (dd, $J_{6'a,6'b} = 14.0$ Hz, $J_{6'a,5'} = 7.5$ Hz)	3.22 (dd, $J_{6'a,6'b} = 13.0$ Hz, $J_{6'a,5'} = 7.5$ Hz)	0.18
	6' _b	3.29-3.23 (m) [3.25]	3.51-3.37 (m) [3.49]	0.24
	NH-2'	×	8.30 (d, $J_{NH,2'} = 8.0$ Hz)	
	NH-1	×	8.24-8.14 (m) [8.20]	
	CH ₃ C=O on 2'	×	2.05 (s)	
	CH ₃ C=O on 1	×	1.97 (s)	

^aThe chemical shift were established based on ¹H, zTOCSY, gCOSY, and gHSQC NMR (500 MHz). ^bCould be analogous position of the 2-deoxystreptamine (DOS) ring. ^cMultiplicity and *J* are given in (). ^dThe numbers in [] were determined from gCOSY and/or zTOCSY. ^eCould be analogous position of the DOS ring. ×Indicates that the acetyl moiety is not present in the molecule.

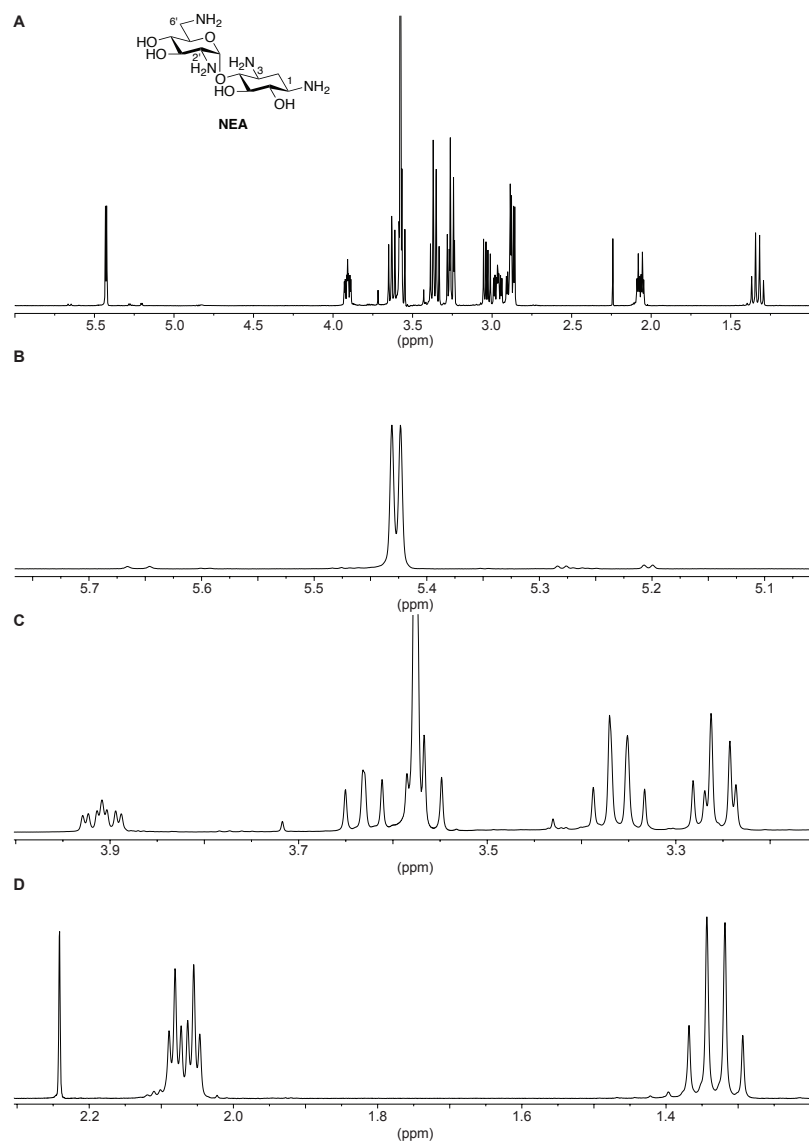


Fig. S5. ^1H NMR of NEA in 1.2:8.8/ $\text{D}_2\text{O}:\text{H}_2\text{O}$ at pH 7.5 (500 MHz). The full spectrum is shown in panel **A** and the expansions in panels **B-D**.

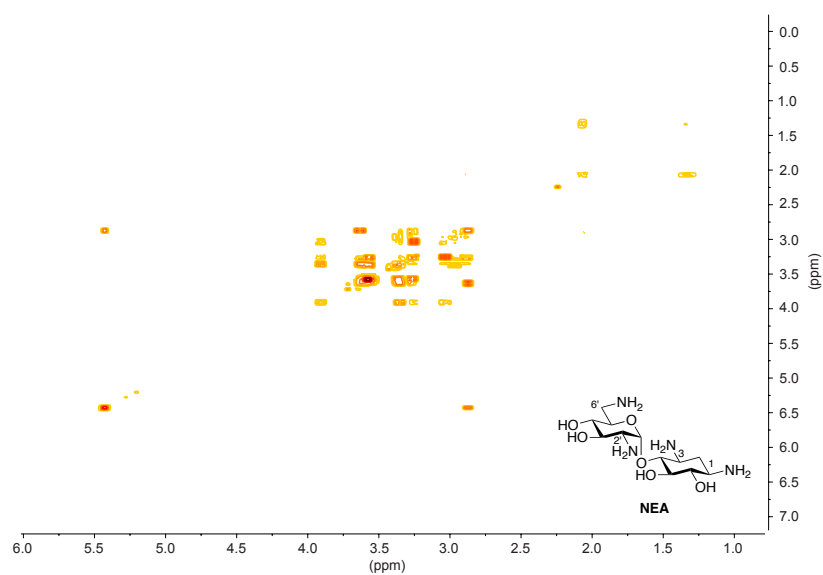


Fig. S6. gCOSY of NEA in 1.2:8.8/D₂O:H₂O at pH 7.5 (500 MHz).

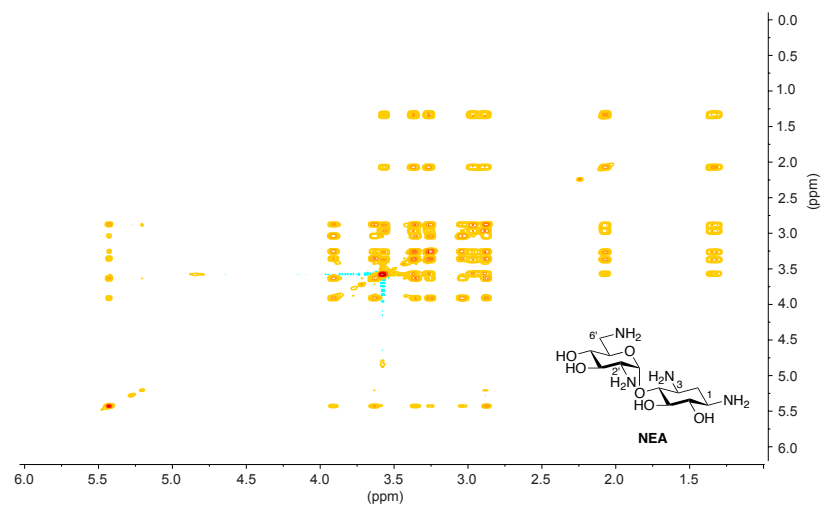


Fig. S7. zTOCSY of NEA in 1.2:8.8/D₂O:H₂O at pH 7.5 (500 MHz).

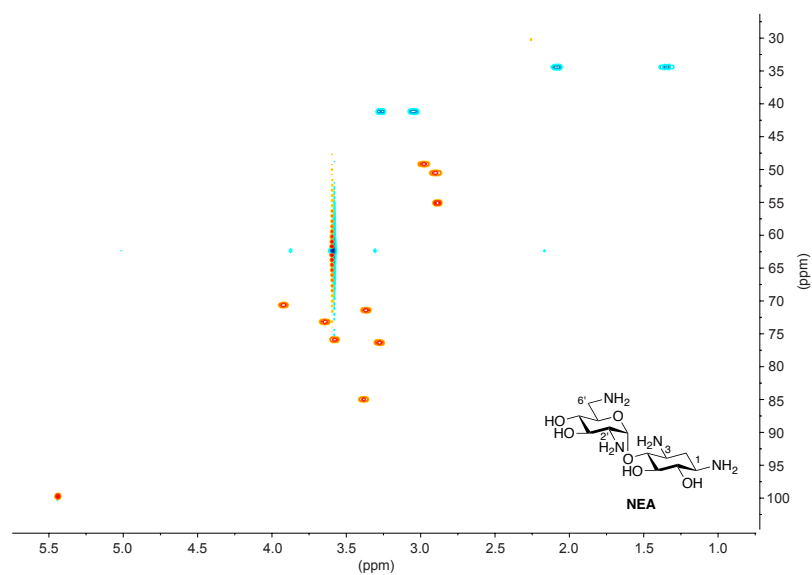


Fig. S8. gHSQC of NEA in 1.2:8.8/D₂O:H₂O at pH 7.5 (500 MHz).

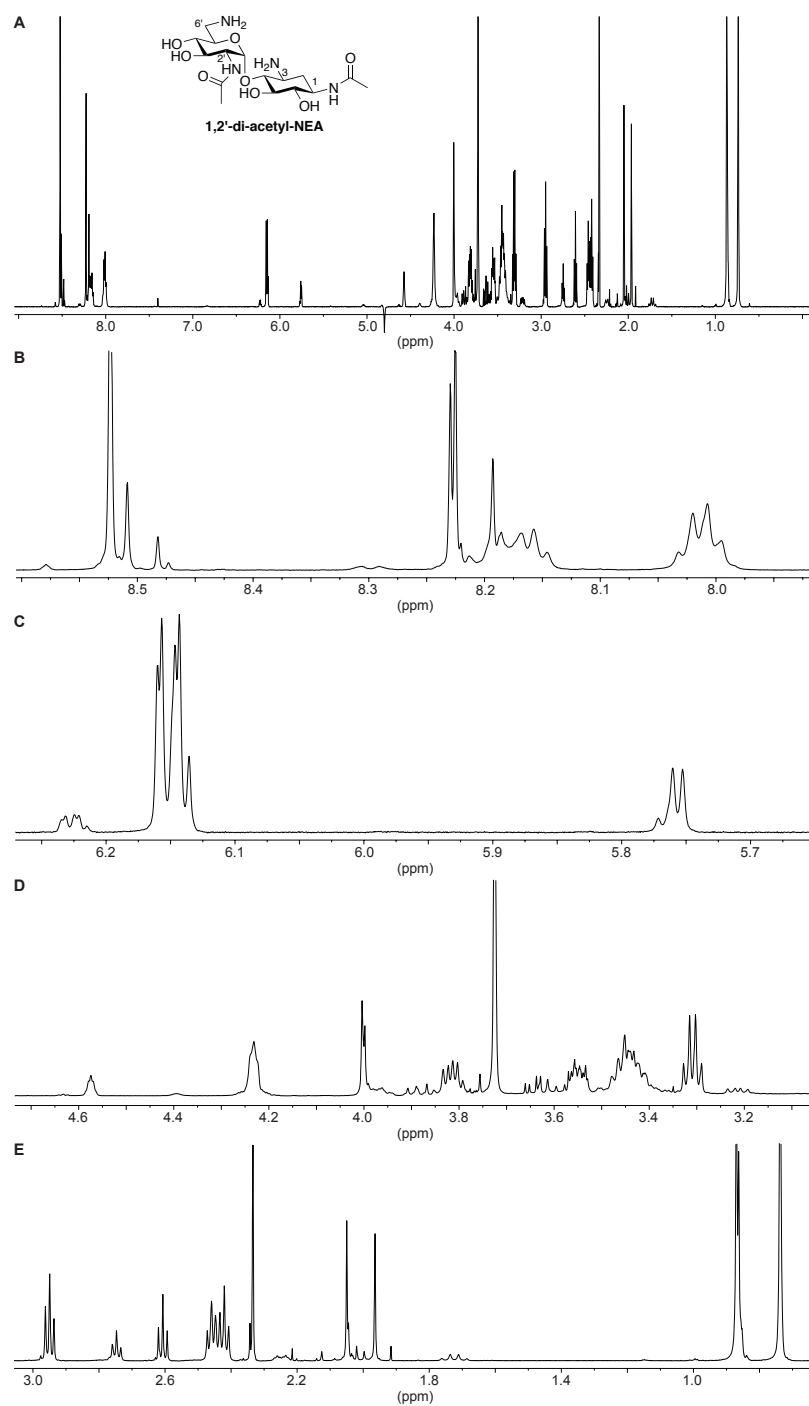


Fig. S9. ^1H NMR of 1,2'-di-acetyl-NEA in 1.2:8.8/ $\text{D}_2\text{O}:\text{H}_2\text{O}$ at pH 7.5 (500 MHz). The full spectrum is shown in panel A and the expansions in panels B-D.

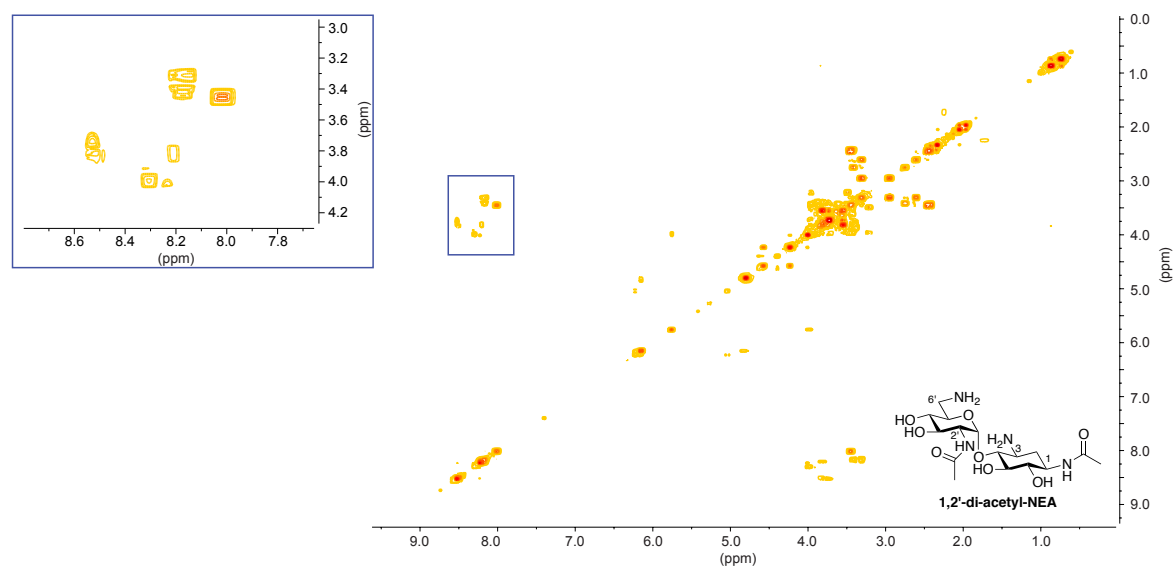


Fig. S10. gCOSY of 1,2'-di-acetyl-NEA in 1.2:8.8/D₂O:H₂O at pH 7.5 (500 MHz).

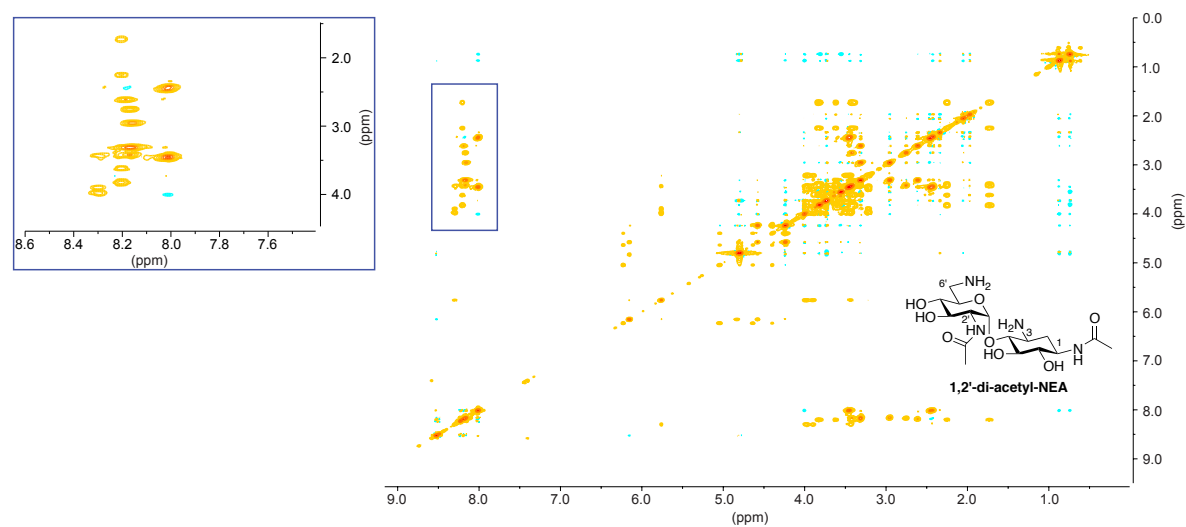


Fig. S11. zTOCSY of 1,2'-di-acetyl-NEA in 1.2:8.8/D₂O:H₂O at pH 7.5 (500 MHz).

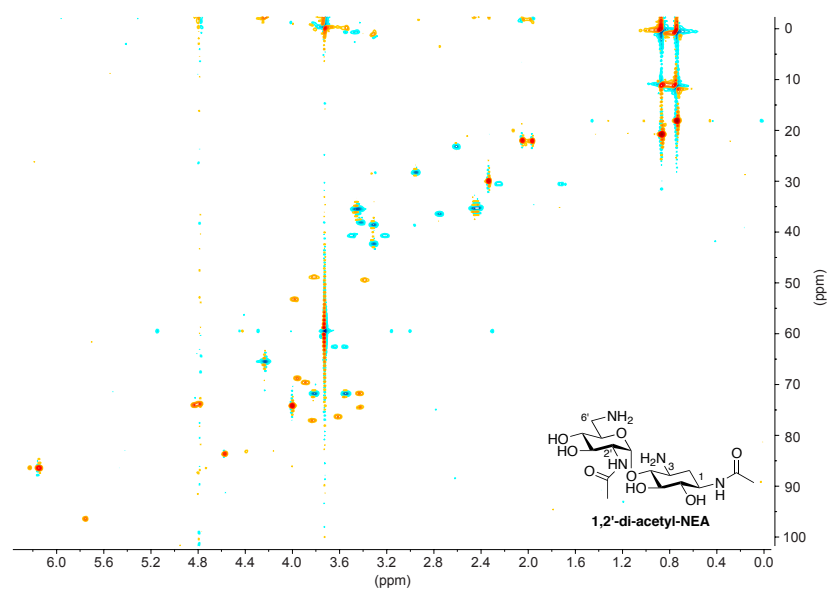


Fig. S12. gHSQC of 1,2'-di-acetyl-NEA in 1.2:8.8/D₂O:H₂O at pH 7.5 (500 MHz).